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(54) Titre : UTILISATION DE LA FAMILLE GENIQUE SGK POUR DIAGNOSTIQUER ET POUR TRAITER LA CATARACTE ET LE GLAUCOME

(54) Title: USE OF THE SGK GENE FAMILY FOR DIAGNOSIS AND THERAPY OF CATARACTS AND GLAUCOMA

(57) Abrégé/Abstract:
The invention relates to the use of a functional inhibitor of hsgk1or hsgk3 protein or a negative transcription regulator of the hsgk1 or hsgk3 gene in the production of a medicament for the treatment and/or prophylaxis of a cataract, glaucoma or diabetic neuropathy. Another aspect of the invention relates to the use of a single-stranded or double-stranded nucleic acid comprising the hsgk1 sequence according to Acc No. NM 005627 or one of the fragments thereof or comprising the hsgk3 sequence according to Acc. No. AF169035 or one of the fragments thereof in the diagnosis of a predisposition to the formation of a cataract, glaucoma and/or diabetic neuropathy, in addition to a kit for diagnosis of a predisposition to the formation of a cataract, glaucoma and/or diabetic neuropathy, comprising the above-mentioned nucleic acid. The invention further relates to various screening methods for identifying and characterizing therapeutically effective substances from a plurality of test substances for the treatment and/or prophylaxis of at least one disease selected from cataracts, glaucoma or diabetic neuropathy.

Abstract

The invention relates to the use of a functional inhibitor of the hsgk1 protein or the hsgk3 protein or of a negative regulator of the transcription of the hsgk1 gene or hsgk3 gene for producing a pharmaceutical for the therapy and/or prophylaxis of a cataract, of a glaucoma or of diabetic neuropathy.

In another aspect, the invention relates to the use of a single-stranded or double-stranded nucleic acid encompassing the hsgk1 sequence according to Acc No. NM_005627, or of one of its fragments, or encompassing the hsgk3 sequence according to Acc No. AF169035, or of one of its fragments, for diagnosing a predisposition for developing cataract, glaucoma and/or diabetic neuropathy, as well as to a kit for diagnosing a predisposition for developing cataract, glaucoma and/or diabetic neuropathy, which kit comprises the abovementioned nucleic acid.

The invention also relates to different screening methods for identifying and characterizing therapeutically active substances, from among a multiplicity of test substances, with the therapeutically active substances being used for the therapy and/or prophylaxis of at least one disease selected from cataract, glaucoma and diabetic neuropathy.

As originally filed

Use of the sgk gene family for diagnosing and treating cataract and glaucoma

The invention relates to the use of a functional inhibitor of the hsgk1 protein or the hsgk3 protein or of a negative regulator of the transcription of the hsgk1 gene or hsgk3 gene for producing a pharmaceutical for the therapy and/or prophylaxis of a cataract, of a glaucoma or of diabetic neuropathy.

In another aspect, the invention relates to the use of a single-stranded or double-stranded nucleic acid encompassing the hsgk1 sequence according to Acc No. NM_005627, or of one of its fragments, or encompassing the hsgk3 sequence according to Acc No. AF169035, or of one of its fragments, for diagnosing a predisposition for developing cataract, glaucoma and/or diabetic neuropathy, as well as to a kit for diagnosing a predisposition for developing cataract, glaucoma and/or diabetic neuropathy, which kit comprises the abovementioned nucleic acid.

The invention furthermore relates to different screening methods for identifying and characterizing therapeutically active substances, from among a multiplicity of test substances, with the therapeutically active substances being used for the therapy and/or prophylaxis of at least one disease selected from cataract, glaucoma and diabetic neuropathy.

The serum and glucocorticoid-inducible kinase hsgk1 was originally cloned as a glucocorticoid-sensitive gene [Webster et al. Characterization of sgk, a novel member of the serine/threonine protein kinase gene family which is transcriptionally induced by glucocorticoids and serum. Mol Cell Biol 1993; 13:2031-2040].

Subsequent investigations revealed that hsgk1 is under the influence of a large number of stimuli [Lang F, Cohen P. Regulation and physiological roles of serum- and glucocorticoid-induced protein kinase isoforms. Science STKE. 2001 Nov 13;2001(108):RE17] such as, inter alia, that of the mineralocorticoids [Chen et al. Epithelial sodium channel regulated by aldosterone-induced protein sgk. Proc Natl Acad Sci USA 1999;96:2514-2519, Náray-Fejes-Tóth et al. sgk is an aldosterone-induced kinase in the renal collecting duct. Effects on epithelial Na^+ channels. J Biol Chem 1999;274:16973-16978; Shigaev et al. Regulation of sgk by aldosterone and its effects on the epithelial Na(+) channel. Am J Physiol 2000;278:F613-F619; Brenan FE, Fuller PJ. Rapid upregulation of serum and glucocorticoid-regulated kinase (sgk) gene expression by corticosteroids in vivo. Mol Cell Endocrinol. 2000;30;166:129-36; Cowling RT, Birnboim HC. Expression of serum- and glucocorticoid-regulated kinase (sgk) mRNA is up-regulated by GM-CSF and other proinflammatory mediators in human granulocytes. J Leukoc Biol. 2000;67:240-248].

hsgk1 is stimulated by the insulin-like growth factor IGF1, by insulin and by oxidative stress by means of phosphoinositol-3-kinase (PI3 kinase) and phosphoinositol-dependent kinase PDK1 by way of a signal cascade [Park et al. Serum and glucocorticoid-inducible kinase (SGK) is a target of the PI 3-kinase-stimulatd signaling pathway, EMBO J 1999;18:3024-3033; Kobayashi et al. Characterization of the structure and regulation of two novel isoforms of serum- and glucocorticoid-induced protein kinase. Biochem. J. 1999;344:189-197]. The activation of hsgk1 by PDK1 involves a phosphorylation at the serine at position 422. The mutation of this serine into an aspartate (^{S422D}SGK1) results in a kinase which is constitutively active [Kobayashi T, Cohen P: Activation of serum- and glucocorticoid-regulated protein kinase by agonists

that activate phosphatidylinositide 3-kinase is mediated by 3-phosphoinositide-dependent protein kinase-1 (PDK1) and PDK2. Biochem J. 1999;339:319-328].

As earlier investigations have shown, hsgk1 is a potent stimulator of the renal epithelial Na^+ channel [De la Rosa et al. The serum and glucocorticoid kinase sgk increases the abundance of epithelial sodium channels in the plasma membrane of Xenopus oocytes. J Biol Chem 1999;274:37834-37839; Böhmer et al. The Shrinkage-activated Na^+ Conductance of Rat Hepatocytes and its Possible Correlation to rENaC. Cell Phys Biochem. 2000;10:187-194; Lang et al. Deranged transcriptional regulation of cell volume sensitive kinase hSGK in diabetic nephropathy. Proc Natl Acad Sci USA 2000;97:8157-8162]. Since hsgk1 is found in a large number of tissues which do not express the epithelial Na^+ channel ENaC, the function of hsgk1 ought not to be restricted to that of regulating the Na^+ channel [Klingel et al. Expression of the cell volume regulated kinase h-sgk in pancreatic tissue. Am J Physiol (Gastroint. Liver-Physiol.) 2000;279:G998-G1002; Waldegger et al. Cloning and characterization of a putative human serine/threonine protein kinase transcriptionally modified during anisotonic and isotonic alterations of cell volume. Proc Natl Acad Sci USA 1997;94:4440-4445; Waldegger et al. *h-sgk* Serine-Threonine protein kinase gene as early transcriptional target of TGF-β in human intestine. Gastroenterology 1999;116:1081-1088].

Due to the fact that hsgk1 probably regulates, in manners which are yet to be elucidated, a large number of other signal transduction pathways or components of these pathways, hsgk1 and its human homologs ought to have considerable potential for diagnosing a large number of diseases. It is evident, in particular, from DE 197 08 173 A1 that hsgk1 can be used for diagnosis

in connection with many diseases, such as hypernatriamia, hyponatriamia, diabetes mellitus, renal insufficiency, hypercatabolism, hepatic encephalopathy and microbial or viral infections, in which changes in cell volume play a crucial pathophysiological role.

WO 00/62781 reported that hsgk1 activates the endothelial Na^+ channel, resulting in renal Na^+ resorption being increased. Since this increase in renal Na^+ resorption is accompanied by hypertension, it was presumed, in this document, that an increase in the expression of hsgk1 would lead to hypertension while a decrease in expression of hsgk1 would ultimately lead to hypotension.

DE 100 421 37 also reported a similar connection between the overexpression or hyperactivity of the human homologs hsgk2 and hsgk3 and hyperactivation of the ENaC, the increase in renal Na^+ resorption which results therefrom, and the hypertension which develops from this. Furthermore, this document already discussed the diagnostic potential of the hsgk2 and hsgk3 kinases with regard to arterial hypertension.

WO 02/074987 A2 disclosed the connection between the occurrence of two different polymorphisms (single nucleotide polymorphism (SNP)) of individual nucleotides in the hsgk1 gene and a genetically determined predisposition for hypertension. These polymorphisms are a polymorphism in intron 6 (T→C) and a polymorphism in exon 8 (C→T) in the hsgk1 gene.

Because sgk1 is expressed in a large number of tissues, and because sgk1 presumably has a large number of yet unknown substrates, it can be expected that there will be further correlations between the function of the human homologs of the sgk family, in particular of the hsgk1 gene (NM_005627), of the hsgk2 gene and of the hsgk3 gene (AF169035) and the development of other

diseases. The uncovering of these other specific disease correlations involving sgk1 could lead to nucleic acids which contain polymorphic regions of the genes of the human homologs of the sgk family, which regions influence the function or expression of the corresponding sgk proteins, being used for diagnosing a predisposition for these other diseases.

The object of the invention was therefore to discover further correlations between the function of the human homologs of the sgk family and new diseases and, in this way, to provide novel possibilities for the diagnostic use of nucleic acids which contain polymorphic regions of the genes of the human homologs of the sgk family.

This object was achieved by means of the surprising finding that hsgk1 and hsgk3 powerfully stimulate the glucose transporter Glut1 (see Fig. 1). Inter alia, the glucose transporter Glut1 mediates the uptake of glucose into various cells of the eye, inter alia [Busik et al. Glucose-induced activation of glucose uptake in cells from the inner and outer blood-retinal barrier. Invest Ophthalmol Vis Sci. 2002;43:2356-63; Takata K, Kasahara T, Kasahara M, Ezaki O, Hirano H. Ultracytochemical localization of the erythrocyte/HepG2-type glucose transporter (GLUT1) in the ciliary body and iris of the rat eye. Invest Ophthalmol Vis Sc. 1991;32:1659-66]. Water follows the glucose osmotically, which means that an increase in the activity of Glut1 leads to cell swelling. Consequently, an increase in the activity of Glut1 could lead to the development of cataract [Gong et al. Development of cataractous macrophthalmia in mice expressing an active MEK1 in the lens. Invest Ophthalmol Vis Sci. 2001;42:539-48]. In addition to this, it has been shown that overexpression of Glut1 promotes the formation and deposition of connective tissue proteins [Ayo et al. Increased extracellular

matrix synthesis and mRNA in mesangial cells grown in high-glucose medium. Am J Physiol. 1991;260:F185-191; Heilig et al. Overexpression of glucose transporters in rat mesangial cells cultured in a normal glucose milieu mimics the diabetic phenotype. J Clin Invest. 1995;96:1802-1814]. Such a deposition of connective tissue proteins impedes the escape of ocular fluid and leads to pressure increases in the eye and consequently to damage of the retina [Fingert et al. Evaluation of the myocilin (MYOC) glaucoma gene in monkey and human steroid-induced ocular hypertension. Invest Ophthalmol Vis Sci. 2001;42(1):145-52, Ueda et al. Distribution of myocilin and extracellular matrix components in the juxtacanalicular tissue of human eyes. Invest Ophthalmol Vis Sci. 2002;43:1068-76]. Glucocorticoids which stimulate the expression of SGK1 (see above) do indeed at the same time lead to the development of glaucoma [Fingert et al. 2001]. However, hsgk1 has never previously been suspected of having a causal role.

The abovementioned disturbances would occur in connection with any situations in which the activity of hsgk1 was increased, that is in the presence of an excess of any of the abovementioned hormones. Particular polymorphisms of the hsgk1 gene which correlate with an increase in blood pressure [Busjahn et al. Serum- and glucocorticoid-regulated kinase (SGK1) gene and blood pressure. Hypertension 40(3): 256-260, 2002] could at the same time lead to an increase in the occurrence of cataract and glaucoma. The same modifications of the gene ought also to correlate with cataract and/or glaucoma which appears prematurely.

The present findings reveal a completely novel mechanism in the regulation of the glucose transporter Glut1. An increase in activity of hsgk1 ought therefore to lead to an increase in the uptake of glucose into

the cells. The transcription of hsgk1 is stimulated by serum [Webster et al. 1993], by glucocorticoids [Brenan & Fuller 2000, Webster et al. 1993], by mineralocorticoids [Chen et al. 1999, Naray-Fejes-Toth et al. 1999, Shigaev et al. 2000, Brennan and Fuller 2000, Cowling and Birnboim 2000], by gonadotropins [Alliston et al. Follicle stimulating hormone-regulated expression of serum/glucocorticoid-inducible kinase in rat ovarian granulosa cells: a functional role for the Sp1 family in promoter activity. Mol Endocrinol. 1997;11:1934-1949; Alliston et al. Expression and localization of serum/glucocorticoid-induced kinase in the rat ovary: relation to follicular growth and differentiation. Endocrinology. 2000;141:385-395; Gonzalez-Robayna et al. Follicle-Stimulating hormone (FSH) stimulates phosphorylation and activation of protein kinase B (PKB/Akt) and serum and glucocorticoid-Induced kinase (Sgk): evidence for A kinase-independent signaling by FSH in granulosa cells. Mol Endocrinol. 2000;14:1283-1300, Richards et al. Ovarian cell differentiation: a cascade of multiple hormones, cellular signals, and regulated genes. Recent Prog Horm Res. 1995;50:223-254], and by a number of cytokines [Lang & Cohen 2001], in particular by TGF-β [Fillon S. et al. Expression of the Serine/Threonine kinase hSGK1 in chronic viral hepatitis. Cell Physiol Biochem 2002;12:47-54; Lang et al. 2000, Waldegger et al. 1999, Wärntges S et al. Excessive transcription of the human serum and glucocorticoid dependent kinase hSGK1 in lung fibrosis. Cell Physiol Biochem 2002,12:135-142]. In addition to this, the transcription of hsgk1 is increased by cell shrinkage, as shown by the Waldegger et al. 1997 paper which has already been cited. An increase in glucose concentration, as occurs in diabetes mellitus, stimulates the expression of hsgk1 by cell shrinkage and/or by an increase in the formation of TGF-β [Lang et al. 2000]. The expressed hsgk1 is activated by insulin-like growth factor IGF1,

by insulin or by oxidative stress [Kobayashi & Cohen 1999, Park et al. 1999, Kobayashi et al. 1999].

According to the findings in accordance with the invention, the increased expression of hsgk1 increases the activity of the glucose transporter Glut-1. As a result, more glucose is taken up into the cells and the water which subsequently follows by osmosis causes the cells to swell. This is the way in which water is incorporated to an increased extent into the cornea and lens, with this leading, by means of a reduction in transparency, to cataract [Gong et al. 2001].

Glaucoma could also develop in a similar manner and, in addition, by the incorporation of connective tissue [Fingert et al. 2001].

Cell swelling is also suspected to be the cause in the case of diabetic neuropathy [Burg et al., Sorbitol, osmoregulation, and the complications of diabetes. J Clin Invest 1988;81:635-40]. However, an increase in Glut1 activity is to be expected not only in diabetes mellitus but also under the influence of glucocorticoids or in patients exhibiting a genetically determined hyperactivity of hsgk1 [Busjahn et al., Serum- and glucocorticoid-regulated kinase (SGK1) gene and blood pressure. Hypertension 40(3): 256-260, 2002]. Glucocorticoids do indeed give rise to glaucoma [Fingert et al. 2001]. The mechanism responsible for the development of glaucoma in connection with glucocorticoid administration had not previously been known. In particular, it had not previously been known that hsgk1 plays a role in this mechanism and is therefore suitable for use as a target protein for diagnosing and treating a glaucoma.

The observations according to the invention consequently surprisingly demonstrate that hsgk1 and

hsgk3 increase nonepithelial glucose transport as well as increasing the epithelial Na^+ channel. As a result, hsgk1 and hsgk3 have been revealed to possess completely novel pathophysiological significances which should entail important diagnostic and therapeutic/prophylactic consequences.

The invention consequently relates to the use of a functional inhibitor of the hsgk1 protein or the hsgk3 protein or of a negative regulator of the transcription of the hsgk1 gene or hsgk3 gene for reducing cell swelling.

The invention furthermore relates to the use of a functional inhibitor of the hsgk1 protein or the hsgk3 protein or of a negative regulator of the transcription of the hsgk1 gene or hsgk3 gene for producing a pharmaceutical for the therapy and/or prophylaxis of a cataract, a glaucoma or diabetic neuropathy.

This functional inhibitor of the hsgk1 protein or the hsgk3 protein can be a chemical substance of any nature which inhibits the normal physiological activity of the hsgk1 protein or of the hsgk3 protein. The functional inhibitor of the hsgk1 protein or the hsgk3 protein is preferably a low molecular weight chemical substance (a "small molecule") or a protein or peptide. The functional inhibitor of the hsgk1 protein or the hsgk3 protein can, in particular, be an antagonist of these enzymes which blocks the substrate-binding site of the hsgk1 protein or the hsgk3 protein but which, at the same time, is not accessible to any catalytic conversion by the hsgk1 or hsgk3. Antagonists which are suitable in this case are preferably those molecules which are structurally similar to the natural substrate of the hsgk1 protein or of the hsgk3 protein, that is, in particular, which are structurally similar to the phosphorylatable amino acids serine and threonine.

Staurosporine and chelerythrine are two known functional inhibitors of hsgk1. In a particularly preferred embodiment, either staurosporine or chelerythrine is therefore used, as a functional inhibitor of hsgk1 or hsgk3, for the therapy and/or prophylaxis of at least one of the diseases cataract, glaucoma and diabetic neuropathy.

A negative regulator of the transcription of the hsgk1 gene or the hsgk3 gene is defined as a substance which activates the expression of the hsgk1 gene or the hsgk3 gene at the transcriptional level.

In addition to the actual active compound, i.e. the functional inhibitor of hsgk1 or hsgk3 or the negative regulator of the transcription of hsgk1 or hsgk3, the pharmaceutical according to the invention for the therapy and/or prophylaxis of a cataract, of a glaucoma or of diabetic neuropathy can also comprise stabilizers and/or carrier substances, such as starch, lactose, stearic acid, fats, waxes, alcohols or other additives such as preservatives, dyes or flavorings.

The pharmaceutical can be administered in any manner, in particular orally in the form of tablets, granules or capsules or as a solution. Other particularly suitable administration forms concern direct administrations (e.g. on the skin or the eye) in the form of ointments, tinctures or sprays or any type of injection (e.g. subcutaneous or intravenous) or infusion.

The invention furthermore relates to the use of a single-stranded or double-stranded nucleic acid encompassing the hsgk1 sequence according to Acc No. NM_005627, or of one of its fragments, for diagnosing a predisposition for developing cataract, glaucoma and/or diabetic neuropathy. The hsgk1 fragment which the single-stranded or double-stranded nucleic acid can

encompass in this connection is at least 10 nucleotides/base pairs in length, preferably at least 15 nucleotides/base pairs in length, and, in particular, at least 20 nucleotides/base pairs in length.

In this connection, the single-stranded or double-stranded nucleic acid preferably encompasses at least one polymorphic nucleotide of the hsgk1 gene, in particular a single nucleotide polymorphism (SNP) of the hsgk1 gene.

In a particularly preferred embodiment, the single-stranded or double-stranded nucleic acid encompasses, in this connection, at least one of the following SNPs of the hsgk1 gene:

- a G insertion at position 732/733 in intron 2 of the hsgk1 gene,
- the T/C substitution at position 2071 in intron 6 of the hsgk1 gene (WO 02/074987 A2),
- the T/C substitution at position 2617 in exon 8 of the hsgk1 gene (WO 02/074987 A2).

The abovementioned single-stranded or double-stranded nucleic acids can preferably be used to detect the above SNPs of the hsgk1 gene in the genomic DNA or cDNA of the patient by means of the following methods:

- by means of directly sequencing the genomic DNA or cDNA using the above nucleic acids,
- by means of specifically hybridizing the genomic DNA or cDNA with the above nucleic acids,
- by means of a PCR oligonucleotide elongation assay or by means of a ligation assay.

In this connection, the genomic DNA or cDNA of the patient is preferably isolated from a body sample taken from the patient, in particular from saliva, blood,

tissue or cells.

It is to be assumed that the activity of the expressed hsgk1 gene depends on the version of this polymorphism in the hsgk1 gene of the patient and that, consequently, nucleic acids which contain at least one of these polymorphisms are particularly well suited for diagnosing a predisposition for developing cataract, glaucoma and/or diabetic neuropathy.

The invention furthermore relates to the use of a single-stranded or double-stranded nucleic acid encompassing the hsgk3 sequence according to Acc No. AF169035, or of one of its fragments, for diagnosing a predisposition for developing cataract, glaucoma and/or diabetic neuropathy. The hsgk3 fragment which the single-stranded or double-stranded nucleic acid can encompass in this connection is at least 10 nucleotides/base pairs in length, preferably at least 15 nucleotides/base pairs in length and, in particular, at least 20 nucleotides/base pairs in length.

In this connection, the single-stranded or double-stranded nucleic acid preferably encompasses at least one polymorphic nucleotide of the hsgk3 gene, in particular a single nucleotide polymorphism (SNP) of the hsgk3 gene.

In addition to the abovementioned single-stranded or double-stranded nucleic acids, particular antibodies which are directed against substrates of the human homologs of the sgk family, in particular against substrates of hsgk1 and hsgk3, are also suitable for diagnosing a predisposition for developing at least one of the diseases cataract, glaucoma and diabetic neuropathy. These diagnostic antibodies are preferably directed against an epitope of the human homologs of the sgk family (in particular of hsgk1 and hsgk3) which contains the phosphorylation site of the substrate

either in phosphorylated form or in unphosphorylated form.

For example, an overexpression of the hsgk1 protein arising due to the individual genetic makeup of the hsgk1 gene could lead to an increase in the conversion of substrates of the hsgk, i.e. to an increase in the enzymatic phosphorylation of the substrates by the hsgk1. At the same time, the overexpression of the hsgk1 protein would lead to stimulation of the glucose transporter Glut1, with this ultimately bringing about a high level of glucose uptake into the cells of the eye and, subsequently, a high level of water uptake by osmosis and, as a result, ultimately bringing about predisposition for developing cataract, glaucoma and diabetic neuropathy. Detecting the more frequent phosphorylation of hsgk1 substrates by means of an antibody which is directed against a region of the substrate in question which contains the phosphorylation site of the hsgk1 in phosphorylated form or unphosphorylated form could consequently represent a method for diagnosing a predisposition for developing cataract, glaucoma and diabetic neuropathy.

In a preferred embodiment, the ubiquitin protein ligase Nedd4-2 (Acc No. BAA23711) is employed as the substrate of the human homolog of the sgk family. This ubiquitin protein ligase is a protein which is specifically phosphorylated by the human homologs of the sgk family [Debonneville et al., Phosphorylation of Nedd4-2 by Sgk1 regulates epithelial Na(+) channel cell surface expression. EMBO J., 2001;20:7052-7059; Snyder et al., Serum and glucocorticoid-regulated kinase modulates Nedd4-2-mediated inhibition of the epithelial Na(+) channel. J. Biol. Chem., 2002, 277: 5-8]. Phosphorylation sites for hsgk1 possess the consensus sequence (R X R X X S/T), where R is arginine, S is serine, T is threonine and X is any arbitrary amino acid. In Nedd4-2 2 (Acc No. BAA23711) there are two

potential phosphorylation sites for hsgk1 with which the abovementioned consensus sequence matches, i.e. the serine at amino acid position 382 and the serine at amino acid position 468.

The abovementioned antibodies for diagnosing a predisposition for developing at least one of the diseases cataract, glaucoma and diabetic neuropathy are therefore preferably directed against the substrate Nedd4-2 and, particularly preferably, against a region of the Nedd4-2 protein having the sequence of the potential phosphorylation site for hsgk1, i.e. the consensus sequence (R X R X X S/T). In particular, these antibodies are directed against Nedd4-2 protein regions which encompass at least one of the two potential phosphorylation sites serine at amino acid position 382 and/or serine at amino acid position 468.

The invention furthermore relates to a kit for diagnosing one of the diseases cataract, glaucoma and diabetic neuropathy, which kit comprises at least one of the following components:

- antibodies which are directed against hsgk1 or hsgk3,

- single-stranded or double-stranded nucleic acids which are able to hybridize, under stringent conditions, with the hsgk1 gene according to Acc No. NM_005627 or with the hsgk3 gene according to Acc No. AF169035; in particular those single-stranded or double-stranded nucleic acids which encompass polymorphic nucleotides, in particular "SNPs" of the hsgk1 gene or of the hsgk3 gene,

- antibodies which are directed against a substrate of a human homolog of the sgk family; preferably antibodies which are directed against the phosphorylation site of this substrate in the

phosphorylated or unphosphorylated form; in particular antibodies which are directed against the phosphorylation site of Nedd4 or Nedd4-2 in the phosphorylated or unphosphorylated form.

The invention also relates to a screening method for identifying and characterizing therapeutically active substances, from among a multiplicity of test substances, with the therapeutically active substances being used for the therapy and/or prophylaxis of at least one disease selected from the group comprising cataract, glaucoma and diabetic neuropathy, comprising the following steps:

a) Heterologously coexpressing
   i) the glucose transporter Glut1 and
   ii) hsgk1 and/or hsgk3
   in cells,

b) culturing at least one cell aliquot A_1 to A_X in the presence of in each case at least one test substance, with the at least one test substance in each case differing in dependence on the index 1 to X of the cell aliquot, and culturing a control cell aliquot B in the absence of any test substance,

c) determining the activity of the glucose transporter Glut1 in the cell aliquots A_1 to A_X as compared with the activity of the glucose transporter Glut1 in the control cell aliquot B.

A substance library, preferably a small molecular library, or else a protein library or the like, can be employed as the "multiplicity of test substances".

In step a), suitable cells, preferably mammalian cells or cell lines, in particular human cells or cell lines,

are transfected with suitable expression vectors, which contain suitable expression cassettes for expressing Glut1 and hsgk1 and/or hsgk3, using standard methods such as electroporation, CaPO4 precipitation, lipofection or the like. The expression cassettes contain the genomic DNA or the cDNA of the relevant target gene (Glut1, hsgk1 or hsgk3) under the control of suitable promoters which are active in the cell type in question and which are able to express the target gene in a suitable quantity. The expression vectors can additionally contain selection markers.

The transfected cells are then cultured under conditions which enable the target genes i) and ii) to be expressed.

In step b), the transfected cells from a) are divided up into different cell aliquots A_1 to A_X and into a control cell aliquot B. The cell aliquots A_1 to A_X are cultured in the presence of in each case at least one test substance. The test substance(s) which is/are in each case added to the cell aliquots A_1 to A_X differ from each other (in dependence on the index 1 to X of the respective cell aliquot A_1 to A_X). On the other hand, the control cell aliquot B is cultured in the absence of any test substance.

In step c), the activity of the glucose transporter Glut1 in the cell aliquots A_1 to A_X is determined quantitatively in comparison with the activity of the glucose transporter Glut1 in the control cell aliquot B. A test substance which is able to functionally inhibit hsgk1 or hskg3, or which reduces their expression, must have been added to the cell aliquots A_1 to A_X in which a markedly lower value is measured for the Glut1 activity than that which is measured in the control cell aliquot B. Such a substance could be suitable for treating at least one of the diseases cataract, glaucoma and diabetic neuropathy.

In an alternative embodiment, the screening method according to the invention for identifying and characterizing therapeutically active substances, from among a multiplicity of test substances, with the therapeutically active substances being used for therapy and/or prophylaxis of at least one disease selected from the group comprising cataract, glaucoma and diabetic neuropathy, comprises the following steps:

d) Heterologously coexpressing
   i) the glucose transporter Glut1 and
   ii) hsgk1 and/or hsgk3
   in at least one aliquot A_1 to A_X of cells, and
   heterologously expressing
   i) the glucose transporter Glut1
   in at least one aliquot B_1 to B_X of cells

e) culturing the cell aliquots A_1 to A_X and B_1 to B_X in the presence of in each case at least one test substance, with the at least one test substance in each case differing in dependence on the index 1 to X of the cell aliquots,

f) carrying out a comparative determination of the activities of the glucose transporter Glut1 in the cell aliquots A_1 to A_X and in the cell aliquots B_1 to B_X.

The explanations which are given above with regard to the individual procedural steps a) to c) apply in a corresponding manner to the procedural steps d) to f) of the alternative screening method according to the invention.

The invention is explained in more detail by the following Fig. 1.

The uptake of 2-deoxyglucose (in pmol/l/10 min/oocyte)

(arithmetic means ± SEM) is plotted on the ordinate A of Fig. 1. *Xenopus laevis* oocytes were injected with Glut-1 cRNA with or without SGK1, SGK2, SGK3 or protein kinase B (PKB) cRNA (see Example 1).

Fig. 1 shows the increase in the uptake of 2-deoxy-glucose which occurs in oocytes which are expressing hsgk1 or hsgk3 in addition to Glut1 as compared with oocytes which are expressing Glut1 on its own. This thereby demonstrates that the functions of hsgk1 and hsgk3 efficiently stimulate the activity of the glucose transporter Glut1. A similar effect is not seen in the case of oocytes which are expressing hsgk2 or PKBmut instead of hsgk1 or hsgk3.

The invention is explained in more detail by means of the following example.

Example 1: Expression in Xenopus laevis oocytes and two-electrode voltage clamp

Normal SGK1 cRNA [Waldegger S, Barth P, Raber G, Lang F: Cloning and characterization of a putative human serine/threonine protein kinase transcriptionally modified during anisotonic and isotonic alterations of cell volume. Proc Natl Acad Sci USA 1997;94:4440-4445] and constitutively active SGK1 (^{S422D}SGK1) cRNA [Kobayashi & Cohen 1999], as well as normal Glut1 cRNA [Iserovich P, Wang D, Ma L, Yang H, Zuniga FA, Pascual JM, Kuang K, De Vivo DC, Fischbarg J. Changes in glucose transport and water permeability resulting from the T310I pathogenic mutation in Glut1 are consistent with two transport channels per monomer. J Biol Chem. 2002;277:30991-7] were synthesized *in vitro*. The dissection of the *Xenopus laevis* ovaries and the collection and treatment of the oocytes have already been described in detail [Wagner CA, Friedrich B, Setiawan I, Lang F, Bröer S: The use of Xenopus laevis oocytes for the functional characterization of

heterologously expressed membrane proteins. Cell Physiol Biochem 2000;10:1-12]. The oocytes were injected with 5 ng of human Glut1, 7.5 ng of human ^{S422D}SGK1 and/or 5 ng of *Xenopus* Nedd4-2. Control oocytes were injected with water. The uptake of radioactively labeled glucose was measured at room temperature for 2 days after the injection of the respective cRNAs. The control bath solution contained 96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$ and 5 mM HEPES, pH 7.4. All the substances were used at the given concentrations. The final solutions were titrated to pH 7.4 with HCl or NaOH.

Calculations

The data are given as arithmetic means ± SEM; n is the number of oocytes which were examined. All the experiments were carried out in at least three different groups of oocytes. The results were tested for significant differences using Student's *t*-test. Only results in which $P < 0.05$ were regarded as being statistically significant.

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<120> Verwendung der sgk-Genfamilie zur Diagnose und zur Therapie von Karakt und Glaukom

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As enclosed to IPER

Amended Patent Claims

1. The use of an antagonist of the hsgk1 protein or the hsgk3 protein, which antagonist is structurally similar to a natural substrate of the hsgk1 protein or the hsgk3 protein, or the use of a negative regulator of the transcription of the hsgk1 gene or hsgk3 gene, for producing a pharmaceutical for the therapy and/or prophylaxis of a cataract, of a glaucoma or of diabetic neuropathy.

2. The use of an antagonist of the hsgk1 protein or the hsgk3 protein as claimed in claim 2, wherein this antagonist is structurally similar to the phosphorylatable amino acid serine or threonine.

3. A pharmaceutical comprising an antagonist of the hsgk1 protein or the hsgk3 protein, which antagonist is structurally similar to a natural substrate of the hsgk1 protein or the hsgk3 protein, or comprising a negative regulator of the transcription of the hsgk1 gene or the hsgk3 gene, for the therapy and/or prophylaxis of a cataract, of a glaucoma or of diabetic neuropathy.

4. The pharmaceutical comprising an antagonist of the hsgk1 protein or the hsgk3 protein as claimed in claim 3, wherein this antagonist is structurally similar to the phosphorylatable amino acid serine or threonine.

5. The use of a single-stranded or double-stranded nucleic acid encompassing the sequence of a human homolog of the hsgk family, or of one of its fragment, for diagnosing a predisposition for developing cataract, glaucoma and/or diabetic

neuropathy.

6. The use as claimed in claim 5, characterized in that the single-stranded or double-stranded nucleic acid encompasses the sequence of the hsgk1 gene according to Acc No. NM_005627, or of one of its fragments.

7. The use as claimed in claim 6, characterized in that the hsgk1 gene encompasses at least one polymorphic nucleotide, in particular an "SNP" of the hsgk1 gene.

8. The use as claimed in claim 7, characterized in that the polymorphic nucleotide of the hsgk1 gene is selected from the group comprising the G insertion at position 732/733 in intron 2 of the hsgk1 gene, the T/C substitution at position 2071 in intron 6 of the hsgk1 gene and the T/C substitution at position 2617 in exon 8 of the hsgk1 gene.

9. The use as claimed in claim 5, characterized in that the single-stranded or double-stranded nucleic acid encompasses the sequence of the hsgk3 gene according to Acc No. AF169035, or of one of its fragments.

10. The use as claimed in claim 9, characterized in that the hsgk1 gene encompasses at least one polymorphic nucleotide, in particular an "SNP" of the hsgk3 gene.

11. The use of an antibody directed against a substrate of a human homolog of the sgk family for diagnosing a predisposition for developing at least one of the diseases cataract, glaucoma and diabetic neuropathy, wherein the antibody is

directed against an epitope of the human homolog which contains the phosphorylation site either in phosphorylated form or in unphosphorylated form.

12. The use as claimed in claim 11, characterized in that the substrate of the human homolog of the sgk family is Nedd4-2 having the Acc No. BAA23711.

13. A kit for diagnosing one of the diseases cataract, glaucoma and diabetic neuropathy, comprising antibodies which are directed against hsgk1 or hsgk3 or comprising nucleic acids which are able to hybridize, under stringent conditions, with the hsgk1 gene according to Acc No. NM_005627 or with the hsgk3 gene according to Acc No. AF169035, or comprising these antibodies and nucleic acids jointly.

14. The kit as claimed in claim 13, characterized in that the nucleic acids are able to hybridize, under stringent conditions, with the DNA regions of the hsgk1 gene according to Acc No. NM_005627 or of the hsgk3 gene according to Acc No. AF169035 which encompass polymorphic nucleotides, in particular "SNPs" of the hsgk1 gene or of the hsgk3 gene.

15. A screening method for identifying and characterizing therapeutically active substances, from among a multiplicity of test substances, with the therapeutically active substances being used for the therapy and/or prophylaxis of at least one disease selected from the group comprising cataract, glaucoma and diabetic neuropathy, comprising the following steps:

a) Heterologously coexpressing

i) the glucose transporter Glut1 and

ii) hsgk1 and/or hsgk3
in cells,

b) culturing at least one cell aliquot A_1 to A_X in the presence of in each case at least one test substance, with the at least one test substance in each case differing in dependence on the index 1 to X, and culturing a control cell aliquot B in the absence of any test substance,

c) determining the activity of the glucose transporter Glut1 in the cell aliquots A_1 to A_X as compared with the activity of the glucose transporter Glut1 in the control cell aliquot B.

16. The screening method as claimed in claim 15, wherein, in step a), the hsgk1 substrate Nedd4-2 is concomitantly coexpressed in addition to, or instead of, the glucose transporter Glut1 and wherein, in step c), the degree of phosphorylation of the hsgk1 substrate Nedd4-2 (Acc No. BAA23711) is determined in the cell aliquots A_1 to A_X in comparison with the control cell aliquot B.

17. A screening method for identifying and characterizing therapeutically active substances, from among a multiplicity of test substances, with the therapeutically active substances being used for the therapy and/or prophylaxis of at least one disease selected from the group comprising cataract, glaucoma and diabetic neuropathy, comprising the following steps:

a) Heterologously coexpressing
   i) the glucose transporter Glut1 and
   ii) hsgk1 and/or hsgk3

in at least one aliquot A_1 to A_X of cells, and heterologously expressing

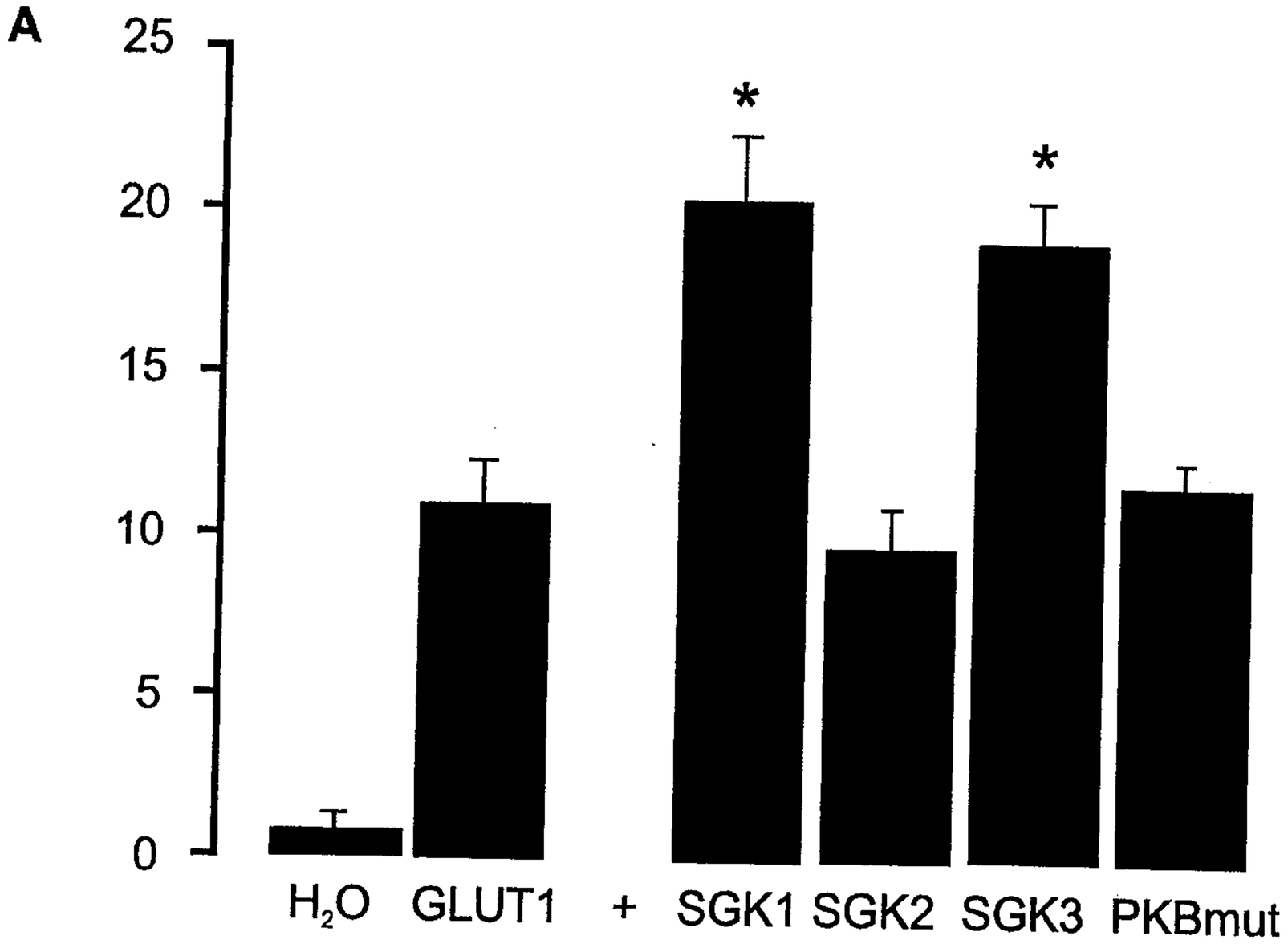
i) the glucose transporter Glut1

in at least one aliquot B_1 to B_X of cells

b) culturing the cell aliquots A_1 to A_X and B_1 to B_X in the presence of in each case at least one test substance, with the at least one test substance in each case differing in dependence on the index 1 to X of the cell aliquots,

c) carrying out a comparative determination of the activities of the glucose transporter Glut1 in the cell aliquots A_1 to A_X and in the cell aliquots B_1 to B_X.

18. The screening method as claimed in claim 17, wherein, in step a), the hsgk1 substrate Nedd4-2 is concomitantly coexpressed, both in the cell aliquots A_1 to A_X and in the cell aliquots B_1 to B_X, in addition to, or instead of, the glucose transporter Glut1 and wherein, in step c), the degree of phosphorylation of the hsgk1 substrate Nedd4-2 (Acc No. BAA23711) is determined in the cell aliquots A_1 to A_X in comparison with the cell aliquots B_1 to B_X.


A
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20
15
10
5
0
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H2O
GLUT1
+
SGK1
SGK2
SGK3
PKBmut